Supplementary Methods

Animals

Vav-iCre mice (obtained from The Jackson Laboratory, strain name B6.Cg-Tg(*Vav1-icre*)$^{A2Kio/J}$) were described previously[1]. The cDNA for exons 8–10 of *Ercc1* along with a neomycin cassette all flanked by *loxP* sites was inserted into the *Ercc1* locus in frame with exon 7 to create a floxed allele of *Ercc1* (Extended Data Fig. 1A). *Ercc1*$^{+/fl}$ FVB/N were crossed with C57BL/6J *Vav-iCre*$^{+/-}$;Ercc1$^{+/-}$ mice to create *Vav-iCre*$^{+/-}$*;Ercc1*$^{-/fl}$ mice carrying one knock-out and one floxed allele excised by codon improved Cre (iCre) recombinase in hematopoietic cells (Extended Data Fig. 1B). WT mice were purchased from Jackson Laboratory. *Ercc1*$^{-/\Delta}$ mice were bred as previously described[2]. *p16*-luciferase reporter mice were obtained from Ohio State University[3]. All experimental mice maintained were in an f1 background from two inbred parents (FVB/n and C57BL/6J) to create congenic mice without strain-specific pathology. Ear punches were used for animal identification and genotyping by TransnetYX (Cordova, TN). Mice were group housed in ventilated micro- isolator cages on Allentown racks. Cage change occurs every two weeks for mice. Animals are handled in a HEPA filtered laminar flow hood with gloves and forceps that are disinfected between cages. All animals are fed irradiated chow (Teklad Global Soy Protein- Free Rodent diet 2020). Bedding and all equipment are autoclaved. Chlorinated water is provided through the Edstrom Reverse Osmosis (RO) automatic watering system supplied to the racks through water manifolds. All animal studies were conducted in compliance with the U.S. Department of Health and Human Services Guide for the Care and Use of Laboratory Animals and were approved by The Scripps Research Institute and University of Minnesota Institutional Animal Care and Use Committee.

RNA isolation and qRT-PCR

Gene expression analysis was performed as described previously[4,5]. Tissues were harvested from euthanized animals and flash frozen in liquid nitrogen. Tissues were homogenized using FastPrep-24 homogenizer (MP Biomedicals, Solon, OH, USA) and total RNA was isolated by Trizol extraction according to manufacturer's specifications (Thermo Fisher, Waltham, MA, USA). Total RNA was quantified using a Nanodrop spectrophotometer (Thermo Fisher) and 1 µg of total RNA used to generate cDNA via the Transcriptor First Strand cDNA synthesis kit (Roche, Basel, Switzerland) according to the manufacturer's specifications. Gene expression changes in *p16*^{Ink4a}*, p21*^{Cip1}*, Il6, Mcp1*, *Tnf*-α were quantified by qRT-PCR reactions using 20 µL reaction volumes and a StepOne thermocycler (Thermo Fisher) with input of 50 ng total RNA per reaction (except *p16*^{Ink4a}, 100 ng total RNA). For each sample, reactions were performed in duplicate. Data was analyzed by ΔΔCt method and the expression was normalized to either *Gapdh*. Primer sequences are as follows: ß2M Fwd 5'-CGGCCTGTATGCTATCCAGA-3', ß2M Rev 5'- GGGTGAATTCAGTGTGAGCC-3'; *Cdkn1a* (*p21*^{Cip1})

Fwd 5'-GTCAGGCTGGTCTGCCTCCG-3', *Cdkn1a* (*p21^{Cip1}*) Rev 5'-CGGTCCCGTGGACAGTGAGCAG-3'; *Cdkn2a* (*p16^{Ink4a}*) Fwd 5'-CCCAACGCCCCGAACT-*3',* *Cdkn2a* (*p16^{Ink4a}*) Rev 5'-GCAGAAGAGCTGCTACGTGAA-3'; *Ercc1* Fwd 5'- AAAAGCTGGAGCAGAACT-3', *Ercc1* Rev 5'-AAGAGCTGTTCCAGGGAT-3'*Gapdh* Fwd 5'-AAGGTCATCCCAGAGCTGAA-3', *Gapdh* Rev 5'-CTGCTTCACCACCTTCTTGA-3'; *Il6* Fwd 5'-CTGGGAAATCGTGGAAT-3', *Il6* Rev 5'-CCAGTTTGGTAGCATCCATC-3'; *Mcp1* Fwd 5'-GCATCCACGTGTTGGCTCA-3', *Mcp1* Rev 5'-CTCCAGCCTACTCATTGGGATCA-3'; *Tnf*-α Fwd 5'-ATGAGAAGTTCCCAAATGGC-3', *Tnf*-α Rev 5'-CTCCACTTGGTGGTTTGCTA-3'; Hmox1 Rev 5'-CTGCTTGTTGCGCTCTATCTC-3'; *Nqo1* Fwd 5'-TGCTATGAACTTCAACCCCA-3', *Nqo1* Rev 5'-GGCGTCCTTCCTTATATGCT-3'; *Nfe2l2* Fwd 5'-GCTTTTGGCAGAGACATTCC-3', *Cat* Fwd 5'-ATAGCCAGAAGAGAAACCCA-3', *Cat* Rev 5'-TTCATGTGCCGGTGACCAT-3'; Firefly Luciferase Fwd 5'-GCCATGAAGCGCTACGCCCTGG-3' Luciferase Rev 5'-TCTTGCTCACGAATACGACGGTGG-3'

Isolation of peripheral blood CD3$^+$ T lymphocytes

CD3$^+$ T lymphocytes was performed as described[5]. Blood was obtained from mice by cardiac puncture, immediately placed into 1/10th volume of 0.5 M EDTA and gently mixed to prevent coagulation. Samples were centrifuged at 2000 rpm for 10 min in a tabletop centrifuge. Supernatant was discarded and the cell pellet was suspended in 1 mL ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1mM Na_2EDTA, pH 7.4) to lyse red blood cells and then incubated at room temperature for 10 min. Cells were spun down and ACK lysis repeated for a second time. Cells were then spun down, washed in 1XDPBS, and resuspended in 1XDPBS with 0.5% FBS and 2 mM EDTA. 50 µL CD3-Biotin conjugate (Miltenyi Biotech, San Diego, CA, USA) were used added to the cell suspension solution and incubated for 30 min on ice. Cells were centrifuged at 1000 rpm for 10 min and washed twice in resuspension buffer. The cell pellet was then resuspended in 500 µL of resuspension buffer and 100 µL of anti-biotin microbeads added before a 15 min incubation on ice. Cells were washed twice and then resuspended in 500 µL of resuspension buffer and applied to MACS column attached to a magnet. Cells were washed with 3X column volume of buffer before elution. Cells were centrifuged and RNA isolation conducted using a RNeasy kit (Qiagen, Germantown, MD, USA) according to manufacturer's specifications. qRT-PCR analysis of senescence markers was performed as indicated above.

Immunoblotting

Snap frozen livers from mice were incubated in RIPA buffer (Thermo-Fisher) on ice for 30 min after being homogenized with a FastPrep-24 homogenizer. Samples were centrifuged at 17,000 x g for 15 min at 4^{o}C. Supernatant was resuspended in 2X SDS loading buffer and 50 µg of total protein run on a 4-15%

SDS-PAGE gel (Bio-Rad, Hercules, CA, USA) before being transferred to nitrocellulose membrane. Membranes were blocked for 1 h in 10% milk TBS-T solution at room temperature before incubation in anti-ERCC1 (Santa Cruz Biotechnology, Santa Cruz, CA, USA, catalog #sc-17089), anti-ɣH2AX (Novus Biologicals, Littleton, CO, USA, catalog# NB100-384, 1:2000) and anti-GAPDH (Abcam, Cambridge, MA, USA, catalog# ab8425, 1:5000) antibody at 4ºC overnight. After washing, samples were incubated in either horse anti-mouse HRP (Cell Signaling Technology, Danvers, MA, USA, catalog #7076S) or goat anti-rabbit HRP secondary antibody (Thermo-Fisher, catalog # 656120, 1:2000) in 5% milk TBS-T solution for 3 h before washing and visualization with ECL (Thermo-Fisher).

Fluorescent in situ hybridization (FISH)

Detection of *p16* mRNA was performed as described previously[5-7]. Briefly, liver, lung and kidney sections were deparaffinized, rehydrated, and boiled in sodium citrate buffer. Slides were prehybridized in a 4X SSC solution containing 3% BSA at 55 °C. Slides were then incubated with either a scrambled non-specific probe or a custom designed *p16* LNA probe (5'-TCTCATGCCATTCCTTTCCTGT-3', Exiqon, Woburn, MA, USA) diluted in hybridization buffer containing 10% dextran sulfate in 4X SSC. Slides were hybridized at 55 °C for 1 h and then submitted to a series of 5 washes of decreasing stringency. Sections were imaged using confocal scanning laser microscopy.

Senescence-associated β-galactosidase (SA-βgal) staining

Fresh tissues from 8-10-month-old *Vav-iCre$^{+/-}$;Ercc1$^{-/fl}$* and littermate controls were fixed in 10% neutral buffered formalin (NBF) for 3-4 h and then transferred to 30% sucrose overnight. Tissues were then embedded in cryo-embedding media (OCT) and cryosectioned at 5 µm for SA-βgal staining (pH 6.0) at 37ºC for 16-24 h in SA-βgal staining solution (40 mM citric acid in sodium phosphate buffer, 5 mM $K_4[Fe(CN)_6]$ $3H_2O$, 5 mM $K_3[Fe(CN)_6]$, 150 mM sodium chloride, 2 mM magnesium chloride and 1 mg/ml X-gal dissolved in N,N-dimethylformamide). Slides were imaged at 20X with a Panoptiq slide scanner (ViewSiq, Vancouver, BC, Canada).

Multiplex analysis of SASP factors

Quantitation of SASP factor abundance was performed as described[5,8]. Serum levels of SASP were measured in *Vav-iCre$^{+/-}$;Ercc1$^{-/fl}$* and littermate controls (n=3-9 mice per group) at different ages using a multiplex assay using the Milliplex Map Mouse Metabolic Hormone Magnetic Bead Panel kit (MCP-1 and TNFα) (Millipore Sigma, St. Louis, MO, USA). 10 µL of serum was analyzed in duplicate and analyte concentrations were quantified on a Luminex 200 (Luminex Corporation, Austin, TX, USA) microplate reader. Serum levels of IL-1ß, Activin A, GDF15, Osteopontin were measured by single-analyte ELISA

(Abcam, Cambridge, MA; R&D Systems, Minneapolis, MN) using a Varioskan plate reader (Thermo-Fisher).

Body Weight and NMR measurement of body composition

A Bruker LF minispec body composition analyzer (Bruker, Billerica, MA, USA) was used to measure body composition of mice (11-25 mice per group). Body weights were measured by use of a standard top loader balance (Ohaus, Parsippany, NJ, USA) and body composition was investigated by using non-invasive nuclear magnetic resonance technique to rapidly measure % fat, lean mass, and fluid in non-anesthetized mice.

Comprehensive blood counts

Tail bleeds from mice were analyzed using a SciL Vet ABC Plus (Henry Schein Animal Health, Gurnee, IL, USA) or HemaTrue (Heska, Loveland, CO) hematology analyzer.

Analysis of functional markers

Serum levels of amylase (pancreatic dysfunction) alanine- (ALT) and aspartate aminotransferase (AST) (liver damage markers) were quantified by ELISA (Abcam) using a Varioskan plate reader (Thermo-Fisher).

Adoptive transfers and *in vivo* imaging detection of luciferase activity

$5x10^6$ bone marrow and splenocytes were harvested from 8-10-month-old *Vav-iCre*$^{+/-}$ and *Vav-iCre*$^{+/-}$*;Ercc1*$^{-/fl}$ and 2-year-old WT mice. Red blood cells were lysed in ACK buffer and then cells were washed in 1XDPBS, and resuspended in 100 µL of 1XDBPS before being retro-orbitally injected into 3-4-month-old isoflurane-anesthetized *p16*$^{Ink4+/Luc}$ mice. Isoflurane-anesthetized mice were subcutaneously injected with 10 µL per gram body weight D-luciferin substrate (Caliper Life Sciences, Hopkinton, MA, USA; 15 mg/ml in 1XDPBS) and were imaged weekly using an IVIS Lumina (PerkinElmer, Waltham, MA, USA) as previously described[3,5].

Immune cell transplantations into progeroid mice

$5x10^6$ bone marrow and splenocytes were harvested from 2-month-old WT mice. Red blood cells were lysed in ACK buffer and then cells were washed in 1XDPBS, and resuspended in 100 µL of DBPS before being retro-orbitally injected into 3-month-old isoflurane-anesthetized *Ercc1*$^{-/\Delta}$ mice. Mice were euthanized one month later, and tissues collected for the senescence marker analysis and circulating SASP factors.

Homing of immune cells in recipient mice

Splenocytes ($10x10^6$ cells) from 7- or 26-month old WT male mice were retro-orbitally injected into 7-month-old female recipient mice. 24 hr after injection tissues were collected and snap frozen in liquid nitrogen. DNA was isolated using Dneasy blood and tissue kit (Qiagen, Germantown, MD) as specified by the manufacturer. Equivalent amounts of total DNA for each sample in a specific tissue was used to amplify the Sry gene by PCR. PCR products were electrophoresed in a gel containing SYBR Safe (Thermo-Fisher) and imaged on an iBright gel imager (Thermo-Fisher).

Sry Fwd 5'-TTGTCTAGAGAGCATGGAGGGCCATGTCAA-3', *Sry* Rev 5'-CCACTCCTCTGTGACACTTTAGCCCTCCGA-3'.

Histopathology

Tissues were collected from euthanized mice and fixed in 10% neutral buffered formalin. Tissues were processed and paraffin embedding before sectioning (4 µm thickness). Sections were stained with hematoxylin and eosin. Specimens were interpreted by a board-certified veterinary pathologist for age-related pathology.

Geropathology

The Geropathology Grading Platform (GGP) is a grading system to assess murine biological aging through the measurement of pathological status of multiple tissues using a standardized scoring system. The scoring system generates a numerical score for the total lesions in each tissue, which are then averaged in each mouse to generate a composite lesion score (CLS)[9].

Delayed-type hypersensitivity measurements

5-month-old *Vav-iCre*$^{+/-}$ and *Vav-iCre*$^{+/-}$*;Ercc1*$^{-/fl}$ mice were sensitized by a 100 µL subcutaneous injection of freshly prepared keyhole limpet hemocyanin (KLH) antigen (2 mg/mL) emulsified 1:1 in Freund's complete adjuvant (Thermo-Fisher). The emulsion was mixed by forcing the adjuvant-immunogen mixture through a small orifice. Two weeks later after sensitization, mice under anesthetized were challenged by injecting 20 ug of KLH (KLH) dissolved in 10 µL of 1XDPBS or 1XDPBS vehicle in rear footpads. The mice were monitored to ensure they regained consciousness before being returned to their cages. Paw thickness of each hind paw was monitored with a spring-loaded caliper (Dyer, Lancaster, PA, USA) at 0, 24, 48 and 72 hr after antigen administration. Only the 0 hr point was measured under anesthesia.

Anti-KLH antibodies ELISA

Serum obtained from mice in the DTH experiments was analyzed for the presence of murine anti-KLH antibodies using the anti-KLH IgG ELISA (Life Diagnostics Inc, West Chester, PA, USA) and a SpectraMax plate reader (Molecular Devices, Sunnyvale, CA, USA). Blood samples were collected from the tail vein.

Peripheral blood and lymphoid analysis by flow cytometry

The spleens (SPL) and lymph nodes (LN) were converted into single cell suspensions and washed with sterile PBS. Red blood cells (RBC) were depleted with RBC lysis buffer (150 mM ammonia chloride, 1 mM sodium bicarbonate, and 0.1 mM EDTA at pH 7.7) and the cells were extensively washed before being passed through a cell strainer. Subsequently, the cells were re-suspended in FACS buffer (2% FBS, 1x PBS, 2mM EDTA, and 0.04% sodium azide) at 3.75 x 10^6 cells per mL. A 200 µL aliquot of each sample was transferred into 96-well polypropylene round-bottom plates (BD Bioscience San Jose, CA, USA). To minimize background noise, Fc receptors were blocked using anti-CD16/CD32 mAb (1:600 dilution; purchased from BD Pharmagin, San Diego, CA, USA) for 20 min at 10°C. The cells were stained with fluorochrome conjugated mAb (purchased from either BD Pharmagin or eBioscience) at the appropriate titer for 45 min at 10° C. The cells were washed with FACS buffer twice and fixed using 2% paraformaldehyde. For intracellular staining, the cells were processed using a cytofix/cytoperm buffer kit purchased from BD Pharmingen (San Diego, CA, USA) and used according to the manufacturer's instructions. Whole blood was collected in heparinized tubes and analyzed as described above after RBC lysis. The samples were processed on a BD LSR II flow cytometer (BD Bioscience San Jose, CA, USA) and analyzed using Flowjo software (Tristar, Inc. Ashland, OR, USA). The absolute number of each cell type was calculated by multiplying the percent calculated by the total splenic cell number.

Sorting of immune cell populations

Bone marrow and splenocytes were harvested from 5-month-old *Vav-iCre*$^{+/-}$ and *Vav-iCre*$^{+/-}$*;Ercc1*$^{-/fl}$ mice. Samples were incubated in ACK lysis buffer to lyse red blood cells before being washed in 1XDPBS before incubating in Fc block for 15 min on ice. Splenocytes were stained with CD3-PE and NK1.1-FITC for 30 min on ice to sort T and NK cells. Bone marrow was stained with CD19-APC, B220-FITC, F4/80-PE-Cy7, and Cd11b-PE purchased from either BD Pharmagin or eBioscience) to sort B cells and macrophages. 5x10^4 cells were sorted into FBS using a BD Aria III flow cytometer. Sorted cells were washed in 1XDPBS and snap frozen in liquid nitrogen. Total RNA was isolated from cells using RNeasy kit and analyzed for the expression of senescence and SASP markers as described above.

Natural killer cell cytotoxicity assay

Spleens collected from 8-12-month-old mice were pressed through a 70 µm filter using a 3 mL syringe filter and rinsed through in 1X DPBS. Samples were centrifuged at 1000 rpm for 10 min in a table top centrifuge. Supernatant was discarded and the cell pellet was suspended in 1 mL ACK buffer (150 mM NH_4Cl, 10 mM $KHCO_3$, 0.1 mM Na_2EDTA, pH 7.4) to lyse red blood cells and then incubated on ice for 5 min. Cells were then spun down, washed in 1XDPBS, and resuspended in 1XDPBS with 0.5% FBS and 2 mM EDTA. 50 µL anti-CD3-Biotin conjugate (Miltenyi Biotech) were used added to the cell suspension solution and incubated for 30 min on ice. Cells were centrifuged at 1000 rpm for 10 min and washed twice in resuspension buffer. The cell pellet was then resuspended in 500 µL of resuspension buffer and 100 µL of anti-biotin microbeads added before a 15 min incubation on ice. Cells were washed twice and then resuspended in 500 µL of resuspension buffer and applied to MACS column attached to a magnet. Column elution fraction was collected and then centrifuged and resuspended and incubated with 50 µL anti-NK1.1-Biotin conjugate on ice for 30 min. Cells were washed with 3X column volume of buffer before elution. Cells were then counted to determine “effector” NK cell numbers for use in cytotoxicity assay. $1x10^6$ K562 “target” cells were incubated in 2 mL of complete media with 20 µL of 3 mM $DiOC_{18}$ stain for 1 h at 37°C. Cells were then washed twice in 1XDPBS to washout remaining $DiOC_{18}$ stain and resuspend in cells in complete media at a density of $1x10^6$ per mL. Suspensions of effector cells were prepared in complete media to yield the following effector:target (E:T) ratios: 15:1, 5:1, and 0:1 by mixing 130 µL of effector cells with 10 µL of target cells. Raise cell volume to 995 µL and add 5 µL of 10 mg/mL DAPI propidium iodide solution and incubate at 37°C for 4 h. Wash cells in 1XDPBS three times and measure specific lysis by flow cytometry.

CyTOF analysis

Maxpar® reagents including water, Cell Staining Buffer (CSB), Cell Acquisition Solution (CAS), Cell-ID Intercalator-Ir, Fix and Perm Buffer and EQ Four Element Calibration Beads were purchased from Fluidigm (South San Francisco, CA, USA). The eBiosciences FoxP3/Transcription Factor staining buffer set was used for fixation/permeabilization and purchased from (ThermoFisher). Paraformaldehyde (PFA) was purchased from EM Sciences and 10X PBS pH 7.2 was purchased from Rockland Immunochemicals (Limerick, PA, USA). Antibodies used for cell surface labeling and phenotyping were either purchased directly from Fluidigm or purchased from the designated manufacturer (Extended Data Table 2). Custom conjugated antibodies were generated in-house through the Mayo Clinic Hybridoma Core using Maxpar X8 Ab labeling kits (Fluidigm) according to the manufacturer’s protocol.

Isolated splenocytes are resuspended in 1 mL of CSB. Each sample was incubated for 5 minutes with 0.5 µm Cisplatin solution in PBS. Samples were then washed twice with CSB. An antibody cocktail

of the extracellular markers was prepared as a master mix prior to adding 50 µL of cocktail to samples resuspended in 50 µL of CSB. Samples were then incubated at room temperature for 45 minutes. After washing twice with CSB, cells were permeabilized with fixation/permeabilization buffer. Afterwards, samples were washed and resuspended in permeabilization buffer before addition of a cocktail of intracellular antibody markers and incubation at room temperature for 45 minutes. Cells were washed and then fixed with 2% PFA for 30 minutes. Cells were then resuspended in intercalation solution and incubated overnight at 4°C. On the following morning cells were washed with PBS and resuspended in a 1:10 solution of calibration beads and CAS at a concentration of $0.5x10^6$ cells/mL. Prior to data acquisition samples were filtered through a 35 µm blue cap tube (Falcon).

Samples were loaded onto a Helios CyTOF® system (Fluidigm) using an attached autosampler and were acquired at a rate of 200-400 events per second. Data were collected as .FCS files using the CyTOF software (Version 6.7.1014). After acquisition intrafile signal drift was normalized to the acquired calibration bead signal using the CyTOF software. CyTOF fcs files were analyzed using Flowjo version 10 using the gating strategy shown in Extended Data 12A-B. Generation of graphs and statistical analysis was performed in Graphpad Prism 8. Statistical significance was determined by performing a non-parametric Kruskal-Wallis test along with Dunn's correction for multiple comparisons. ViSNE analysis was performed using Cytobank software. $CD45^+$ cells from all 19 samples were equally sampled for a total of 1,299,999 events and the analysis was performed with 3,000 iterations and a perplexity of 50. We used 15 channels for the analysis: TCRβ, CD3e, CD62L, CD4, CD8, CD44, GATA3, FOXP3, Tbet, RORγT, CD25, CD19, CD11b, CD11c, NK1.1. viSNE illustrations were generated in Cytobank.

EPR quantitation of $O_2^{\cdot-}$

Spleen tissue (25 mg) was homogenized in ice-cold HBSS pH 7.4 containing 100 mM DTPA. The homogenate was then exposed to the EPR hydroxylamine spin probe CMH (1-Hydroxy-3-methoxycarbonyl-2,2,5,5-tetramethylpyrrolidine), 200 mM, for 10 min at 25 C and then centrifuged at 1000 x g and the supernatant (50 mL) placed into an EMXnano EPR cavity for analysis. Controls were performed with added superoxide dismutase (purified CuZnSOD) to validate signal from $O_2^{\cdot-}$. Values for signal intensity are arbitrary units of signal intensity taken from the up-field peak of the characteristic three-line spectrum from the nitrogen splitting.

Measurement of splenic catalase activity

Catalase activity was measured as previously described[10] by detection of hydrogen peroxide at 240 nm using a Cary 300 BIO UV-VI (Varian, Palo Alto, CA) spectrophotometer at 30s intervals for a total of 1

min. Catalase activity per milligram of protein (*k*/mg) was quantified using the following formula: *k*/mg = [3 ln ($Abs_{initial}/Abs_{final}$)] / [milligrams of protein * time].

Quantitation of 8-oxo-guanine DNA lesions

Tissues from 8-10-month-old mice were analyzed for 8-oxo-guanine (8-oxo-dG) levels, using the ELISA kit (Abcam, Cambridge, MA, USA) according to manufacturer's specifications.

Lipid peroxidation products

Tissues from 8-10-month-old mice were analyzed for 4-hydroxynonenal adducts using the OxiSelect HNE Adduct Competitive ELISA kit (Cell Biolabs, San Diego, CA, USA) according to manufacturer's specifications as described[10].

Quantitation of t glutathione

Fresh tissues from mice were immediately homogenized in 5% sulfosalicylic acid and subsequently analyzed for the concentration of reduced (GSH) and oxidized (GSSG) glutathione using the Glutathione Assay Kit (Cayman Chemical, Ann Arbor, MI, USA) as described[10].

Short course rapamycin administration

3-month-old *Vav-iCre*$^{+/-}$ and *Vav-iCre*$^{+/-}$*;Ercc1*$^{-/fl}$ mice were given intraperitoneal injections of 4 mg/kg rapamycin (LC Laboratories, Woburn, MA, USA) that was formulated with 5% PEG-400 and 5% Tween-80 every other day for six weeks. Mice were given one week for washout before beginning delayed-type hypersensitivity experiments as described above.

β2-microglobulin measurement

Analysis of serum and urinary levels of β2-microglobulin was performed by ELISA (Abcam) as specified by the manufacturer.

Intervertebral disc aggrecan immunofluorescence

Mouse lumbar intervertebral disc tissue were isolated from spines and fixed overnight at 4° C in 2% paraformaldehyde. For immunofluorescent staining, the tissues were cryoprotected with 30% sucrose in PBS overnight at 4° C, then embedded in OCT (Tissue-Tek). Serial axial plane cryosections were cut at thicknesses of 5 µm. The tissue sections were rehydrated in PBS, permeabilized and blocked with 0.25% Triton X-100, 10% goat serum and 1% BSA in PBS for 30 min at room temperature. Incubation with anti-aggrecan (Cat. No. AB1031, Millipore, Burlington, MA) was carried out overnight at 4° C following

blocking. The sections were then incubated with secondary antibodies (Cy3-conjugate Goat anti-rabbit IgG, *Jackson laboratory*) for 60 min at room temperature, according to the manufacturer's protocols. Immunostained sections were imaged and analyzed using a Nikon instrument A1 confocal laser microscope and NIS-elements microscopy imaging software.

1,9-dimethylmethylene blue (DMMB) colorimetric assay for sulfated glycosaminoglycans (GAGs)

For each mouse, NP tissue isolated from four lumbar IVDs of each mouse were pooled and digested using papain at 60°C for 2 hr. GAG content was measured in duplicates by the DMMB procedure using chondroitin-6-sulfate (Millipore Sigma C-8529) as a standard[11]. The DNA concentration of each sample was measured using the PicoGreen assay (Molecular Probes) and used to normalize the GAG values.

Muscle injury

4 μM of CTX (Millipore Sigma, C9759) was injected intramuscularly into the gastrocnemius muscles of the recipient mice. Five days after injury the mice were sacrificed, and the muscles were harvested, flash frozen in liquid nitrogen-cooled 2-methylbutane. Serial 10 μm cryosections were then H&E stained for the identification of injury area. Image acquisition was performed with a Nikon Eclipse Ci at 2-20× magnification. For measurement of injured area: at least 6 random 2x magnification fields were blindly measured with Image J.

Grip strength analysis

Body weights were collected for each mouse and grip strength was measured using a BIO-GS3 grip strength meter (Bioseb, Pinellas Park, FL).

Immunohistochemistry

Cryosections from CTX and non-CTX injured muscle were fixed with 5% formalin, blocked with 5% donkey serum, and then incubated with antibodies specific for CD68 (marker of M1 macrophages, ab53444, 1:200, Abcam) and CD163 (marker of M2 macrophages, Sc-33560, 1:50, Santa Cruz) were used to evaluate the ratio of M1/M2 macrophages as a parameter for inflammation in the muscle. Alexafluor 594-conjugated anti-rabbit IgG (1:500; Invitrogen, A21207) and Alexafluor 488-conjugated anti-rat IgG (1:500; Invitrogen, A21208) were used as secondary antibodies. The nuclei were stained with DAPI. All the stained sections were visualized on a Nikon Eclipse Ni-E fluorescence microscope. Ten random pictures per slide were taken and they were blindly measured with Image J.

Schematics

All mouse images in schematics were adapted from BioRender.

Data availability

Reasonable requests for all data presented in this manuscript will be honored by the corresponding authors.

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